

MRSA transmission dynamics among interconnected acute, intermediate- and long-term healthcare facilities in Singapore

Angela Chow^{1,2}, Vanessa W Lim¹, Ateeb Khan³, Kerry Pettigrew³, David CB Lye^{4,5}, Kala Kanagasabai⁶, Kelvin Phua⁷, Prabha Krishnan⁸, Brenda Ang^{4,5}, Kalisvar Marimuthu^{4,5}, Pei-Yun Hon⁸, Jocelyn Koh⁷, Ian Leong⁹, Julian Parkhill¹⁰, Li-Yang Hsu^{2,4}, Matthew TG Holden³

¹Department of Clinical Epidemiology, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital, Singapore

²Saw Swee Hock School of Public Health, National University of Singapore, Singapore

³School of Medicine, University of St Andrews, St Andrews, Fife, UK

⁴Department of Infectious Diseases, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital, Singapore

⁵Yong Loo Lin School of Medicine, National University of Singapore, Singapore

⁶Renci Hospital, Singapore

⁷Ang Mo Kio-Thye Hua Kuan Hospital, Singapore

⁸Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore

⁹Department of Continuing and Community Care, Tan Tock Seng Hospital, Singapore

¹⁰The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK

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6 **Corresponding author**

7 Angela Chow, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital,
8 11 Jalan Tan Tock Seng, Singapore 308433, Singapore

9 E-mail: Angela_Chow@ttsh.com.sg

10 **Alternate Corresponding author**

11 Matthew Holden, School of Medicine, Medical & Biological Sciences, North Haugh,
12 University of St Andrews, United Kingdom.

13 E-mail: mtgh@st-andrews.ac.uk

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15 **40-word Summary:**

16 MRSA transmission dynamics among interconnected acute, and intermediate and long-
17 term care facilities (ILTCs) varied between clones. Clonal complexes ST22 and ST45
18 successfully spread throughout the healthcare system, and are established in ILTCs.
19 MRSA prevention is critical in ILTCs.

20

1 **Abstract (250 words)**

2 Background

3 Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common healthcare-
4 associated multidrug-resistant organism. Despite the interconnectedness between
5 acute hospitals (AHs) and intermediate- and long-term care facilities (ILTCs), the
6 transmission dynamics of MRSA between healthcare settings is not well understood.

7

8 Methods

9 We conducted a cross-sectional study in a network comprising an AH and five closely-
10 affiliated ILTCs in Singapore. A total of 1,700 inpatients were screened for MRSA over a
11 6-week period in 2014. MRSA isolates underwent whole genome sequencing, with a
12 pairwise SNP (Hamming distance) cutoff of 60 core genome SNPs used to define recent
13 transmission clusters (clades) for the three major clones.

14

15 Results

16 MRSA prevalence in intermediate-care (ITCs) (29.9%) and long-term care facilities (LTCs)
17 (20.4%) were significantly higher than in the AH (11.8%) ($p < 0.001$). The predominant
18 clones were ST22 (183, 47.8%), ST45 (129, 33.7%) and ST239 (26, 6.8%), with greater
19 diversity of STs in ILTCs relative to the AH. A large proportion of the clades in ST22
20 (14/21, 67%) and ST45 (7/13, 54%) included inpatients from the AH and ILTCs. The most
21 frequent source location of the inter-facility transmissions was the AH ($n=28$, 36.4%).

22 Conclusions

1 MRSA transmission dynamics between the AH and ILTCs were complex. The greater
2 diversity of STs in ILTCs suggests that the eco-system in such settings might be more
3 conducive for intra-facility transmission. ST22 and ST45 have successfully established
4 themselves in ILTCs. The importance of interconnected infection prevention and control
5 measures and strategies cannot be overemphasized.

6

7

1 INTRODUCTION

2 Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common
3 healthcare-associated drug-resistant organisms globally, especially in Asia [1]. MRSA has
4 also evolved from being an almost purely healthcare-associated pathogen to one that is
5 increasingly isolated from the community and from livestock, such that the traditional
6 classifications of healthcare-associated MRSA (HA-MRSA) and community-associated
7 MRSA (CA-MRSA) are progressively blurred [2]. There remain relatively few successful
8 HA-MRSA clones that have spread globally. In Asia, the major successful HA-MRSA
9 clones belong to multilocus sequence type (ST) 239, ST5 (New York-Japan clone), ST22
10 (UK-EMRSA-15) [1,3], and lately ST45 [4-6].

11 HA-MRSA emerged in Singapore in the late 1970s [7]. Between the late 1980s
12 and 2000, virtually all HA-MRSA were ST239 [3,7]. This changed with the importation of
13 ST22 MRSA around 2000, which became the dominant HA-MRSA clone by 2010 [3,7-9].
14 Phylogenetic analysis of representative isolates of both HA-MRSA clones across three
15 major acute care hospitals (AHs) in Singapore revealed interdependent evolution for
16 both clones, suggesting that frequent exchanges of patients and staff had occurred
17 between the hospitals[3]. Since 2010, a third global clone of HA-MRSA,ST45, has
18 appeared in Singaporean hospitals and is increasing in prevalence [5].

19 Besides horizontal transfers between AHs, vertical transfers of patients from AHs
20 to intermediate- and long-term care facilities (ILTCs) are common in Singapore. Several
21 studies suggesting that ILTCs – with their reduced staff-to-patient ratios and less
22 stringent infection prevention practices – may serve as reservoirs of MRSA within the

1 healthcare system [10-12].

2 Whole genome sequencing (WGS) is fast emerging as the new gold standard for
3 bacterial molecular epidemiology [13-18]. Various studies looking at within-host single
4 nucleotide polymorphism (SNP) diversity and MRSA transmission have defined the cut-
5 off for a recent (i.e. days and weeks rather than months) transmission event as 40 to 60
6 pairwise core genome SNPs (Hamming distance) [16-18]. In examining the spread of
7 ST239 within and between intensive care units of a hospital in northeastern Thailand, a
8 (Hamming) pairwise distance cutoff of 60 SNPs was used to define recent transmission
9 clusters (clades) which were epidemiologically supported. We therefore sought to
10 investigate the MRSA transmission dynamics between an AH and its closely affiliated
11 ILTCs using WGS, defining clades for further analysis via Hamming distance calculations.

12 **METHODS**

13 ***Study design and settings***

14 A cross-sectional study was conducted in a 1,700-bed adult tertiary-care AH in
15 Singapore and its five most closely-affiliated ILTCs: a 105-bed rehabilitation center (ITC
16 1), a 78-bed community hospital (ITC 2), a 235-bed community hospital (ITC 3), a 234-
17 bed nursing home (LTC 1), and a 164-bed chronic sick unit (LTC 2). The study took place
18 over a six-week period from June 2 to July 9 2014. We randomly selected 999 inpatients
19 with >48 hours stay in the AH to participate in the study. All residents of the ILTCs were
20 included.

21 ***Bacterial isolates***

1 Nasal, axillary, and groin swabs were obtained from all study subjects
2 sequentially over the six-week period in order to capture the contemporaneity of MRSA
3 isolates from the various healthcare facilities, as the estimated mutation rate of one
4 core single-nucleotide polymorphism (SNP) for MRSA is approximately every six weeks
5 [3,15]. MRSA was cultured from the swabs, and DNA extracted from the isolates, using
6 conventional methods (see Supplementary Methods for more details).

7 ***Whole genome sequencing and data access***

8 WGS was performed following previously described protocols (Supplementary
9 Methods) [3,15]. Short reads for all sequenced isolates have been submitted to the
10 European Nucleotide Archive (ENA; <http://www.ebi.ac.uk/ena/>) under study accession
11 number PRJEB9390. Individual accession numbers of sequences and assemblies for all
12 isolates are listed in Supplementary Table 1.

13 ***Data analysis***

14 *Quantitative analysis*

15 The differences in MRSA prevalence between healthcare facilities were
16 compared using the Chi-square test, with the odds ratios and 95% confidence intervals
17 (CI) of the associations estimated. Differences in age and duration of stay were
18 compared using the Student's t-test and Wilcoxon rank-sum test respectively. All
19 statistical analyses were performed using Stata version 13 (Stata Corp., College Station,
20 TX).

21 *Bioinformatics and phylogenetic analysis*

1 The sequence reads were aligned against the appropriate reference sequences
2 using SMALT (<http://www.sanger.ac.uk/science/tools/smalt-0>) and SNPs were identified
3 as described previously (Supplementary Methods) [3]. Phylogenetic trees for major
4 clones were constructed using RAxML v7.0.4 [19].

5 *Determination of clades and parsimonious reconstruction of transmissions events*

6 Single isolates were picked as representative of a sample site, and in some
7 individuals there were multiple isolates from different sites. Where isolates were from
8 the same ST, a primary isolate, representative of that individual, was chosen; sites were
9 preferentially picked in the following sequence: nasal, groin, followed by axilla. Isolates
10 belonging to different STs from the same individual were included in the analysis.

11 Hamming distances were calculated (Supplementary Figure 1). This cut-off was
12 then used to define clades for each major ST. For each of the clades, parsimonious
13 reconstruction of transmissions events between the different healthcare locations was
14 conducted using the phylogenetic trees and associated healthcare setting metadata. The
15 basal isolate in each clade was assigned as the origin state, and transmissions
16 parsimoniously reconstructed onto the phylogeny from root to tips to identify inter- and
17 intra-facility transmission events.

18 **Ethics**

19 Ethical approval for the study was obtained from the Domain Specific Research
20 Board, National Healthcare Group.

1 RESULTS

2 There were 1,700 subjects screened for MRSA during the study period, with a
3 participation rate of 86% at the ILTCs. Subjects across the healthcare facilities were
4 similar in terms of age and gender, whereas subjects in LTCs had longer duration of stay
5 compared to subjects in ITCs and the AH (Table 1). The prevalence of MRSA in ITCs
6 (29.9%) and LTCs (20.4%) were significantly higher than in the AH (11.8%) ($p<0.001$).

7 We sequenced 383 MRSA isolates from 289 subjects from the prospective
8 screening. The predominant lineages were ST22 ($n=183$, 47.8%), ST45 ($n=129$, 33.7%),
9 ST239 ($n=26$, 6.8%), and ST1 ($n=18$, 4.7%), with small numbers of STs from other clonal
10 complexes (CCs) ($n=27$, 7.0%). ST22 was more prevalent in the AH (53%) and ITCs (53%)
11 than in the LTCs (31%) ($P= 0.005$) (Figures 1A-C). In contrast, LTCs had the greatest
12 diversity of MRSA clones (Figure 1C). CA-MRSA clones (ST59 and ST30) were observed in
13 an intermediate-care (ST59, $n=2$, 0.5%) and a long-term care facility (ST30, $n=2$, 0.5%)
14 respectively. Seventy-eight subjects (27.0%) had MRSA recovered simultaneously from
15 different body sites, of which 65 (83.3%) had isolates with the same ST, whereas the
16 remainder had isolates belonging to two different STs at different sites.

17 Three major HA-MRSA lineages were the focus of MRSA transmission dynamic
18 investigations: ST22, ST45 and ST239. In total 270 isolates were subject to phylogenetic
19 analysis to elucidate the fine-scale genetic relationships between representative isolates
20 from the subjects in each ST (Figure 2). The ST22 isolates ($n=143$) were differentiated by
21 2775 SNP sites, the ST45 ($n=107$) by 1533 SNP sites, and ST239 ($n=20$) by 637 SNP sites.

1 The healthcare origins of the isolates in relation to phylogenetic relationships
2 were categorized as heterogeneous, with isolates from all six settings distributed
3 throughout the phylogenies (Figure 2). This is consistent with the interconnectivity of
4 the healthcare network. The narrow temporal sampling of this study enabled us to look
5 for evidence of both intra- and inter-facility transmission of MRSA. Isolates that are part
6 of a transmission chain will share a recent common ancestor, and therefore will be
7 phylogenetically linked and genetically similar. In the phylogenies of the three main
8 MRSA populations, there were clusters of isolates from the same healthcare setting
9 suggestive of intra-facility transmission. In addition, there were clusters composed of
10 isolates of mixed origins indicating that inter-facility transmission has occurred.

11 The majority of the subjects had isolates that were found in Hamming defined
12 clades (Figure 2). In the ST22 population, 21 clades were identified comprising 124
13 isolates (86.7%) (Figure 2A), consistent with the distinct clusters observed on the tree.
14 Similarly in the ST45 population, 13 clades comprising 95 isolates (88.8%) (Figure 2B)
15 were identified. In the ST239 population this identified six clades comprising 19/20
16 isolates (95.0%) (Figure 2C).

17 Among the clades, 30 (77%) had at least one patient from the AH. The remaining
18 clades comprised of either ITCs alone ($n=4$), ITCs and LTCs ($n=2$), or LTCs alone ($n=3$).
19 Except for two clades (clade 45_6 and clade 22_12, Supplementary Table 1), at least one
20 subject in each clade had been hospitalized in the study AH or another AH within the
21 preceding 12 months. The other subjects in almost all clades (except clusters 22_15 and

45_3, Supplementary Table 1) who had not had an acute hospitalization episode had shared the same ward with at least one subject with a recent AH hospitalization.

The largest clade was identified in the ST45 population (Cluster 45_12, 40 subjects; Supplementary Table 1), comprising predominantly of patients from the AH, whereas the second largest clade was from the ST22 (clade 22_2, 37 subjects; Supplementary Table 1) which had the majority of patients from ITCs.

A larger proportion of the clades in ST22 (14/21, 67%) compared to ST45 (7/13, 54%) included patients and residents from both the AH and ILTCs. The remaining clades comprised either of patients from the AH or residents from ILTCs. In contrast, 40% (2/5) of the clusters in the ST239 phylogeny comprised of patients from the AH only.

Transmission events within the clades were reconstructed parsimoniously using phylogenetic analyses. In total 193 transition events could be designated (Supplementary Table 2). Over half of the events ($n=116$, 60.1%) were identified as being intra-facility transmissions, with the AH having the largest number ($n=59$) of events, followed by ITC1 ($n=18$), ITC3 ($n=17$), LTC1 ($n=11$), ITC2 ($n=6$) and then LTC2 ($n=5$). Examining the inter-facility transmissions, the most frequent source location of the transmissions was the AH ($n=28$, 36.4%), followed by ITC3 ($n=21$, 27.3%), LTC2 ($n=12$, 15.6%), ITC2, ($n=9$, 1.7%), LTC1 ($n=4$, 5.2%) and then ITC1 ($n=3$, 3.9%). A summary of the transmissions identified in the clades is presented in Figure 3 and illustrates the pathways of transmissions.

DISCUSSION

1 This study provided insights into the population dynamics of MRSA within an
2 interconnected healthcare network of an AH with its closely affiliated ILTCs. MRSA
3 prevalence in AH (11.8%) was significantly lowest compared to intermediate-care (ITCs)
4 (29.9%) and long-term care facilities (LTCs) (20.4%) ($p<0.001$). The predominant clones
5 were ST22 (183, 47.8%), ST45 (129, 33.7%) and ST239 (26, 6.8%), with greater diversity
6 of STs in ILTCs relative to the AH. A large proportion of the clades in ST22 (14/21, 67%)
7 and ST45 (7/13, 54%) consists of inpatients from both the AH and ILTCs. AH was the
8 major source location of inter-facility transmissions ($n=28$, 36.4%).

9 The transmission of MRSA within the network is a complex one.
10 Contemporaneously, different MRSA clones were identified within the same healthcare
11 institution and the same MRSA clade observed across healthcare settings. The higher
12 MRSA prevalence observed in ILTCs relative to the AH in our study was consistent with
13 other studies [10-12], and – combined with the finding of a greater diversity of STs in
14 ILTCs – indirectly suggests that infection prevention practices are less stringent in the
15 ILTCs. The observation that the predominant clonal lineages were ST22, ST45 and
16 ST239 also reflects what was previously reported [5], with the major change being that
17 of the increased prevalence of ST45 vis-à-vis ST239.

18 The Hamming distance defined clusters allowed us to examine the recent
19 dynamics of the MRSA. Our results suggest that the current dominant lineage ST22
20 appeared to have successfully transmitted from acute hospitals to ILTCs. In the ST22
21 phylogenetic tree, isolates from ILTCs interspersed with isolates from the AH within
22 many clades. Furthermore, almost 1 in 5 clades in ST22 included only patients/residents

1 from ILTCs, suggesting that ST22 was being spread independently within ILTCs. The
2 same findings were made for ST45, with a suggestion that isolates from this ST might
3 preferentially spread within ILTCs given that 40% of the clades comprised of isolates
4 obtained only from ILTC subjects. Despite ST239 being the oldest HA-MRSA clone in
5 Singapore, it did not appear to have transmitted as successfully as ST22 and ST45 across
6 healthcare settings. It is unclear if this was the result of ST239 being outcompeted at the
7 ILTCs by the other two STs, or if differential infection control practices at the various
8 healthcare facilities had played a role in the divergent distribution of the STs.

9 Parsimonious reconstruction of transmission events suggests that the AH was
10 the source of MRSA with regards to transmission between the AH and the ITCs;
11 however, it was the reservoir with regards to the transmission from LTCs. This` result
12 seems incongruent with the higher prevalence of MRSA in ILTCs but may be explained
13 by the general flow of patient movements. AH inpatients tend to be transferred to ITCs
14 and are then discharged home, with only a small percentage requiring transfer back to
15 the AH. LTCs on the other hand are the terminal care facility for patients transferred
16 there, who stay until they are deceased or develop an acute event requiring transfer
17 back to the AH. Moreover, far fewer patients are transferred directly from AH to LTC as
18 compared to AH to ITC, or ITC to LTC.

19 Our study was limited by the cross-sectional design and short sampling frame,
20 and the results based on genomic analysis will need validation via a longitudinal study.
21 Second, the overall movement of patients and staff between the healthcare facilities
22 was not evaluated, factors which may inform resolution to the net transmission of

1 MRSA between the various healthcare facilities. A higher participation rate at both the
2 AH and ILTCs would have made for a more rigorous study; however, the results are
3 unlikely to change significantly given the participation rate of 86%.

4 In conclusion, we found that the transmission dynamics of MRSA between an AH
5 and its closely affiliated ILTCs varied between MRSA CCs. ST22 and ST45 have not only
6 receded ST239 in acute hospitals [3,5], but have also successfully established
7 themselves in the ILTCs. The greater diversity of STs in ILTCs suggests that the eco-
8 system in such settings might be more conducive for intra-facility transmission.
9 Interconnected infection prevention and control measures and strategies, including
10 sharing of information on MRSA-colonizers and best practices, should be instituted
11 across acute hospitals and ILTCs in healthcare networks.

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1 **Figures**

2 **Figure 1.**

3 Distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) clones in the A) acute
4 hospital (AH), B) intermediate-care (ITC), and C) long-term care (LTC) facilities, by total
5 number of isolates ($n=383$)

6

7 **Figure 2.**

8 Population structures of the dominant methicillin-resistant *Staphylococcus aureus*
9 (MRSA) clones circulating in health care facilities defined as maximum likelihood
10 phylogenetic trees based on core genome SNPs of: A) ST22 B) ST45; and C) ST239. Also
11 shown (right-hand panels) are: clades of isolates defined by the pairwise 60 SNP cutoff
12 (clusters are alternatingly colored from top to bottom, blue and red) and healthcare
13 facilities. The trees are rooted with the reference used for mapping for each ST. In the
14 case of CA-347, the ST45 reference, the branch has been collapsed. Tree branches
15 colored blue link isolates that belong to a clade (as indicated in the right-hand panel).
16 One ST22 isolate, CD141496, single locus variant of ST22, was excluded from the
17 phylogenetic analysis due to its genetic distance from the rest of the isolates in the
18 collection.

19

20 **Figure 3**

21 Schematic representation of the transmission dynamics of methicillin-resistant
22 *Staphylococcus aureus* (MRSA) in the healthcare settings. For each of the clusters,

1 parsimonious reconstruction of transmissions events between the different healthcare
2 facilities were conducted using the phylogenetic trees and healthcare setting metadata.
3 The origin of the basal isolate in each cluster was assigned as the initial state, and
4 subsequent transmissions parsimoniously reconstructed to identify inter- and intra-
5 facility transmission events. The arrows are scaled in size according to the number of
6 observed inter-facility transmission events, and the circles representing the 6 different
7 healthcare locations are scaled in size according to the number of intra-facility
8 transmission events identified.

9

1 REFERENCES

- 2 1. Molton JS, Tambyah PA, Ang BSP, Ling ML, Fisher DA. The global spread of
3 healthcare-associated multidrug-resistant bacteria: a perspective from Asia. Clin
4 Infect Dis, **2013**; 56: 1310–1318.
- 5 2. Bal AM, Coombs GW, Holden MT, et al. Genomic insights into the emergence and
6 spread of international clones of healthcare-, community- and livestock-associated
7 methicillin-resistant *Staphylococcus aureus*: blurring of the traditional definitions. J
8 Glob Antimicrob Resist, **2016**; 6: 95-101.
- 9 3. Holden MTG, Hsu LY, Kurt K, et al. A genomic portrait of the emergence, evolution,
10 and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic.
11 Genome Res, **2013**; 23: 653–664.
- 12 4. Tsao FY, Kou HW, Huang YC. Dissemination of methicillin-resistant *Staphylococcus*
13 *aureus* sequence type 45 among nursing home residents and staff in Taiwan. Clin
14 Microbiol Infect, **2015**; 21: 451-458.
- 15 5. Hon PY, Koh TH, Krishnan P, et al. Changing molecular epidemiology and high rates
16 of mupirocin resistance among methicillin-resistant *Staphylococcus aureus* in
17 Singaporean hospitals. J Glob Antimicrob Resist, **2014**; 2: 53-55.
- 18 6. Ho PL, Chow KH, Lo PY, Lee KF, Lai EL. Changes in the epidemiology of methicillin-
19 resistant *Staphylococcus aureus* associated with spread of the ST45 lineage in Hong
20 Kong. Diagn Microbiol Infect Dis, **2009**; 64: 131-137.

- 1 7. Hsu LY, Koh TH, Singh K, Kang ML, Kurup A, Tan BH. Dissemination of
2 multisusceptible methicillin-resistant *Staphylococcus aureus* in Singapore. J Clin
3 Microbiol, **2005**; 43: 2923–2925.
- 4 8. Hsu LY, Loomba-Chlebicka N, Koh YL, et al. Evolving EMRSA-15 epidemic in
5 Singapore hospitals. J Med Microbiol. **2007**; 56: 376–379.
- 6 9. Teo J, Tan TY, Hon PY, et al. ST22 and ST239 MRSA duopoly in Singaporean
7 hospitals: 2006-2010. Epidemiol Infect, **2013**; 141: 153–7.
- 8 10. Lee BY, Bartsch SM, Wong KF, et al. The importance of nursing homes in the spread
9 of methicillin-resistant *Staphylococcus aureus* (MRSA) among hospitals. Med Care,
10 **2013**; 51: 205–215.
- 11 11. van den Dool C, Haenen A, Leenstra T, Wallinga J. The role of nursing homes in the
12 spread of antimicrobial resistance over the healthcare network. Infect Control Hosp
13 Epidemiol, **2016**; 37: 761–767.
- 14 12. Garazi M, Edwards B, Caccavale D, Auerbach C, Wolf-Klein G. Nursing homes as
15 reservoirs of MRSA: myth or reality? J Am Med Dir Assoc, **2009**; 10: 414-8.
- 16 13. Parkhill J, Wren BW. Bacterial epidemiology and biology – lessons from genome
17 sequencing. Genome Biol, **2011**; 12: 230.
- 18 14. Cartwright EJP, Köser CU, Peacock SJ. Microbial sequences benefit health now.
19 Nature, **2011**; 471: 578.
- 20 15. Harris SR, Feil EJ, Holden MTG, et al. Evolution of MRSA during hospital
21 transmission and intercontinental spread. Science, **2010**; 327: 469–474.

- 1 16. Long SW, Beres SB, Olsen RJ, Musser JM. Absence of patient-to-patient
2 intrahospital transmission of *Staphylococcus aureus* as determined by whole-
3 genome sequencing. MBio, **2014**; 5: e01692-14.
- 4 17. Price JR, Golubchik T, Cole K, et al. Whole-genome sequencing shows that patient-
5 to-patient transmission rarely accounts for acquisition of *Staphylococcus aureus* in
6 an intensive care unit. Clin Infect Dis, **2014**; 58: 609-618.
- 7 18. Tong SY, Holden MT, Nickerson EK, et al. Genome sequencing defines phylogeny
8 and spread of methicillin-resistant *Staphylococcus aureus* in a high transmission
9 setting. Genome Res, **2015**; 25: 111-118.
- 10 19. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of
11 large phylogenies. Bioinformatics **2014**; 30: 1312–1313

FIGURES

Figure 1-A.

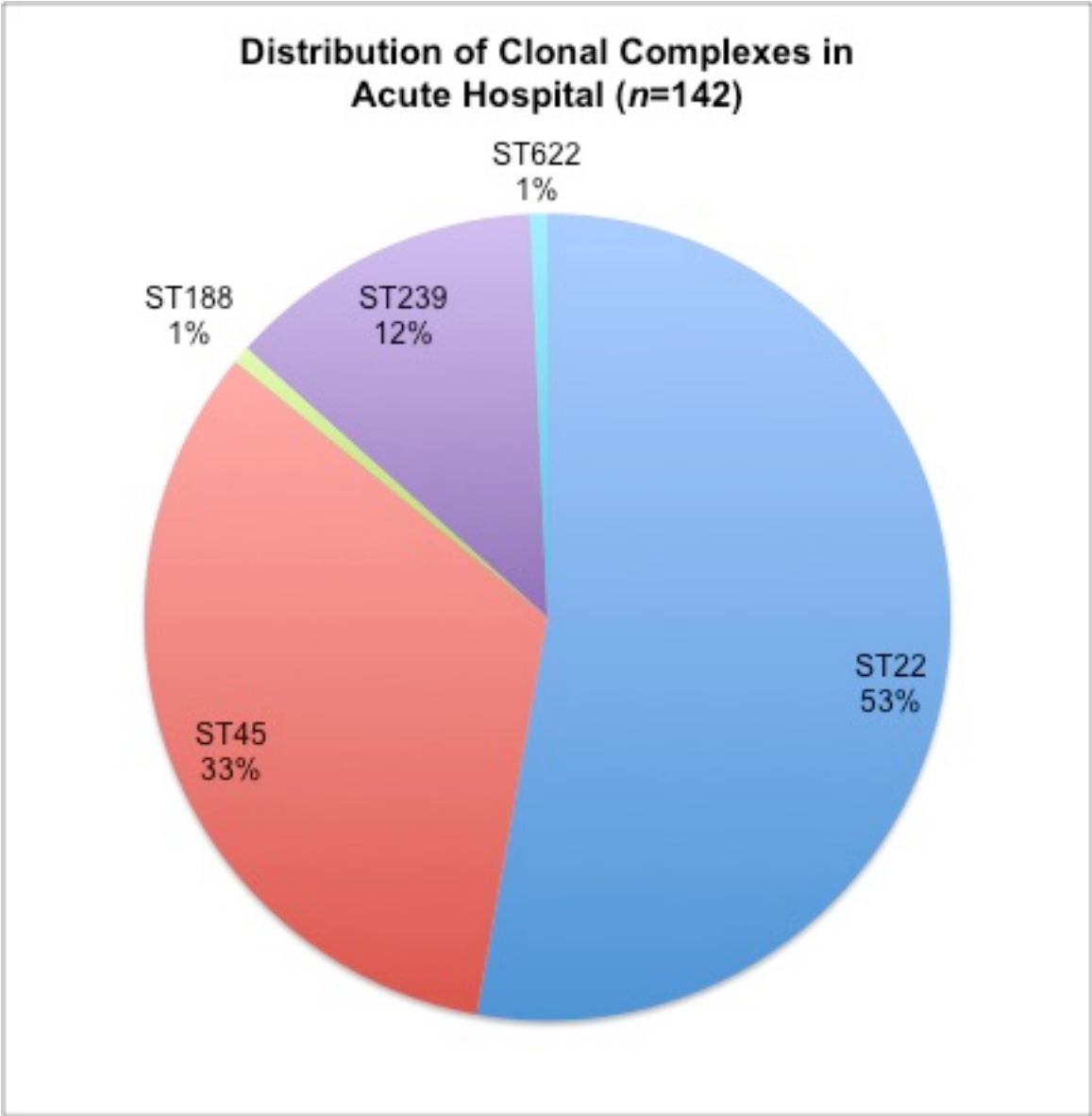


Figure 1-B. Distribution of Clonal Complexes in Intermediate Care Facilities (*n*=118)

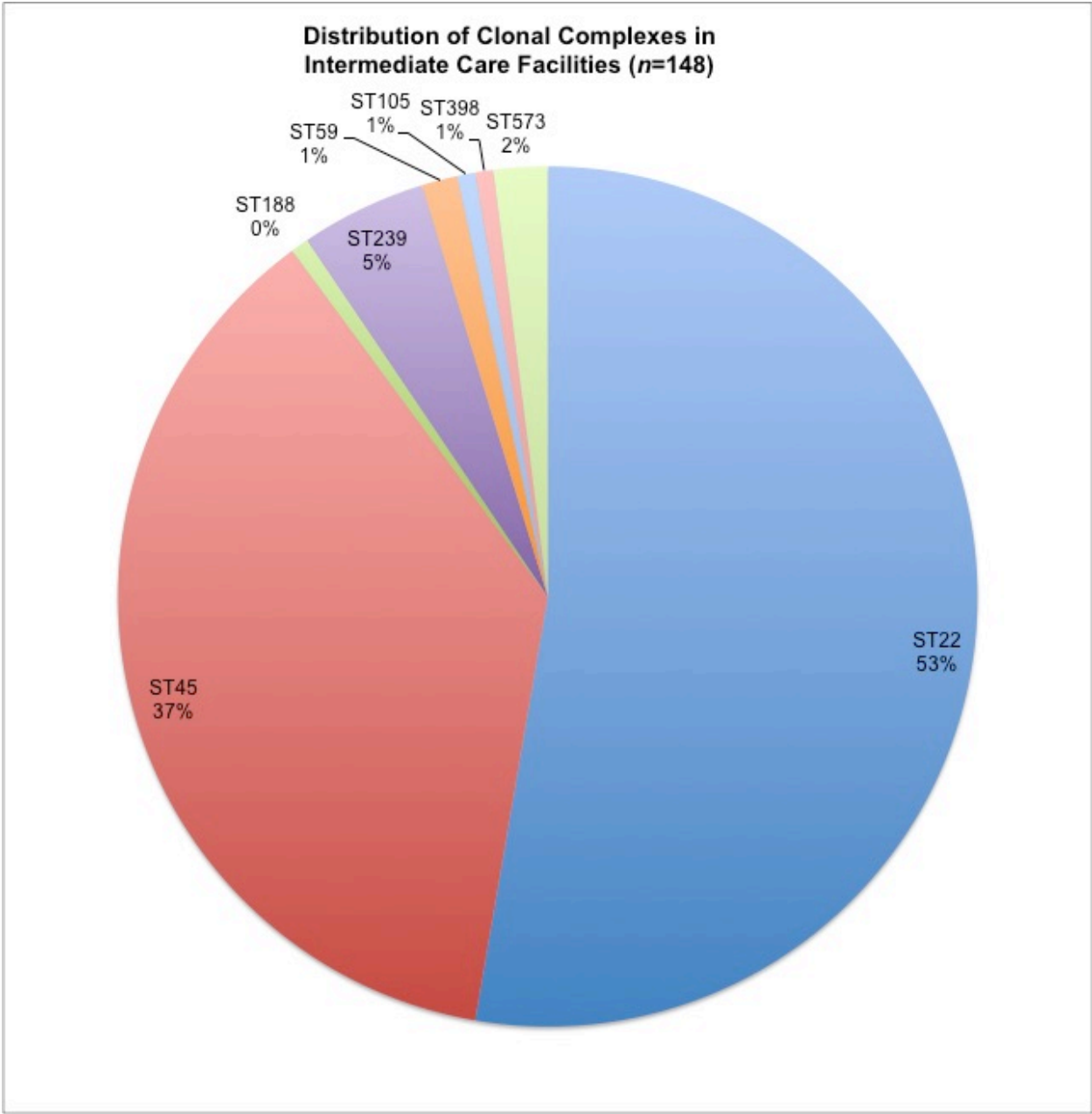
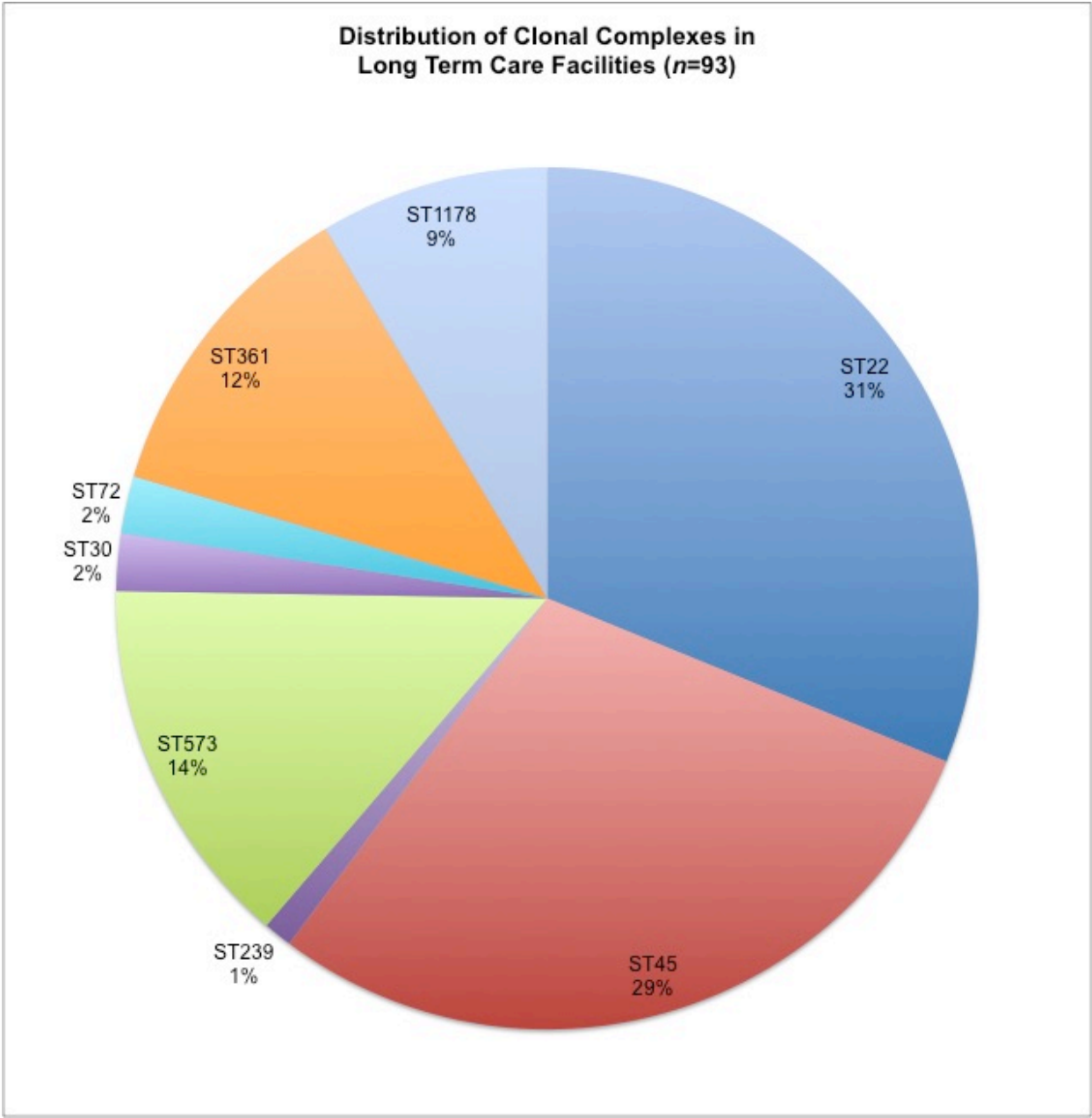


Figure 1-C.



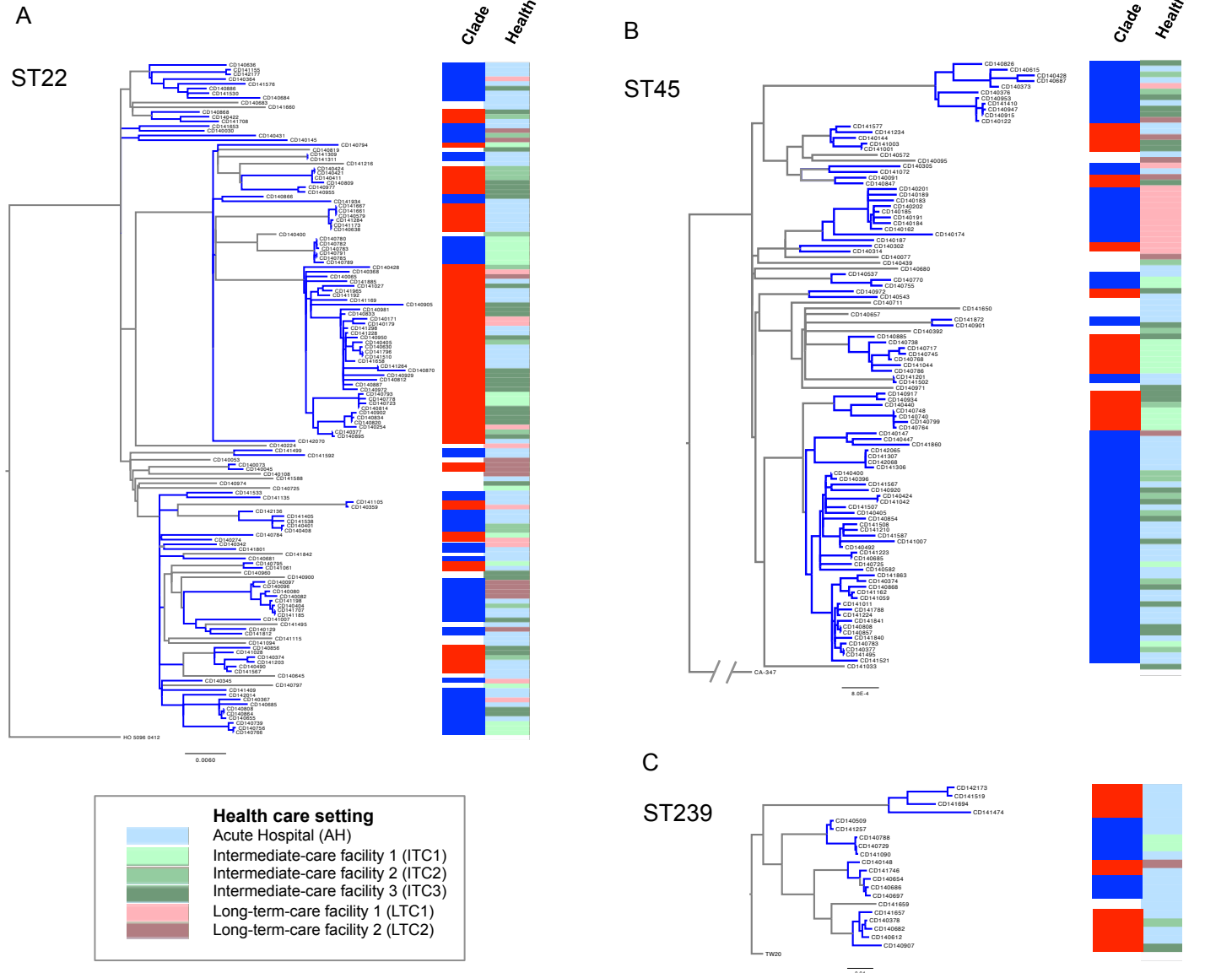


Figure 2.

Figure 3.

